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Size Effect in Molecular Imaging of Vascular Endothelial Growth Factor

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Visualizing and quantifying vascular endothelial growth factor (VEGF) levels are crucial factors in understanding the tumor environment and monitoring antiangiogenic treatment. The small peptidic probes described by Fedorova et al. in this issue appear to be superior to antibodies in reflecting the dynamics of VEGF as they facilitate image quantification.

Angiogenesis is critical in tumor progression, and it is now widely accepted that mutations of oncogenes and tumor suppressor genes can cause a tumor to become angiogenic (Bergers and Benjamin, 2003). Emerging preclinical and clinical evidence (Niu and Chen, 2010) has substantiated that vascular endothelial growth factor (VEGF), a key regulator of tumor angiogenesis, is an effective target for the prevention and control of malignancies, especially for solid tumors. The effectiveness of antiangiogenic drugs, however, does not always translate into changes in tumor size, making the radiological evaluation of efficacy using standard Response Evaluation Criteria In Solid Tumors (RECIST) and/or World Health Organization (WHO) criteria inappropriate (Faivre et al., 2007). Accordingly, there are intensive investigations to develop noninvasive imaging modalities that reflect the functional and molecular changes associated with therapeutic interventions. Molecular imaging probes that target VEGF or the VEGF receptor (VEGFR) could enable new paradigms for the assessment of antiangiogenic treatment and facilitate a better understanding of the role of VEGF/VEGFR expression profiles in many angiogenesis-related diseases. Although VEGF receptors are relatively accessible, it has proven very challenging to image and understand VEGF expression, owing to the solubility and dynamic nature of VEGF.

So far, most VEGF-related imaging studies have focused on immuno-single photon emission computed tomography (SPECT) or immuno-positron emission tomography (PET), which have used imaging probes consisting of radiolabeled antibodies against VEGF. For example, bevacizumab (Avastin; Roche), a humanized monoclonal antibody directed against all VEGF-A isoforms, has been labeled with ^{64}Cu ($t_{1/2}$ = 12.7 h) after DOTA conjugation to image VEGF expression in HT29 colorectal cancer xenografts. PET imaging showed a clear accumulation of ^{64}Cu-DOTA-bevacizumab in the tumors. In addition, tumor accumulation of ^{64}Cu-DOTA-bevacizumab correlated well with VEGF expression, as measured by western blot analysis (Paudyal et al., 2011). In a clinical study of 14 patients with clear cell renal cell cancer (ccRCC) performed by Desar et al. (2010), all five controls (i.e., those not treated with neoadjuvant therapy) showed a preferential tumor accumulation of ^{111}In-bevacizumab.

The expression level of VEGF within tumor regions is much lower than that of many other tumor markers, such as EGFR, HER2, or integrins. Thus, it is ineffective to use VEGF-targeted imaging for direct diagnosis. Rather, a more appropriate application of VEGF imaging has been to monitor therapeutic responses to antiangiogenic treatment (Desar et al., 2010; Nagengast et al., 2011). For example, treatment with sorafenib (Nexavar; Bayer), a small-molecule tyrosine kinase inhibitor against VEGFRs, resulted in a significant decrease of ^{111}In-bevacizumab uptake in ccRCC (Desar et al., 2010).

The change in VEGF levels that is induced by therapeutic drugs is a very dynamic process. Thus, repetitive timely imaging is critical to accurately reflect VEGF levels in tumor environments. Unfortunately, this is difficult to achieve with full-length antibodies that are labeled with long half-lived isotopes. Bevacizumab, for example, is typically labeled with long half-lived radioisotopes to facilitate imaging at up to seven days after tracer injection. To gain more insight into the dynamics of the response of a tumor to antiangiogenic treatment, Nagengast et al. (2011) labeled Ranibizumab, a Fab fragment of bevacizumab, with ^{89}Zr ($t_{1/2}$ = 78.4 h) for PET imaging. Nevertheless, they still needed up to 24 hr to obtain optimal images.

In the current issue of *Chemistry & Biology*, Fedorova et al. (2011) have identified new peptide probes for VEGF imaging using phage display techniques. Their optimized peptide, Z-3B, showed high binding affinity and specificity for VEGF. Unlike common linear peptides, which are very flexible and unstable, Z-3B forms a very stable three helix scaffold structure. The small peptide facilitates labeling with ^{18}F, a radionuclide with a short half-life of 109.8 min. For ^{18}F-Z-3B, the optimal imaging time point is at only 2 hr after tracer injection, compared with a period of 48 to 72 hr for PET imaging with antibodies. Fast renal and hepatobiliary clearance resulted in good tumor-to-blood and tumor-to-muscle ratios of the peptide tracer. For imaging only purposes, particularly in the case of VEGF, small molecules with high binding affinity and specificity would be superior to antibodies and antibody fragments. Theoretically, patients can be re-imaged at very short intervals with such peptidic probes, if wished, to visualize any changes in VEGF expression at a greater time-resolution.

To stratify patient responses to therapy, image quantification is needed to assess target expression or activity. For example,

imaging probe accumulation in certain regions relates mainly to target expression and probe-target interaction. However, other factors—such as blood flow, extravascularization of the probes, and interstitial pressure—also need to be taken into account (Niu et al., 2009). Although ^{111}In-bevacizumab uptake in tumors decreased significantly after treatment with sorafenib, based on immunohistochemistry data, VEGF levels did not change accordingly. These results demonstrated that VEGF expression is not the major parameter that determines bevacizumab accumulation. Other factors such as tumor vessel characteristics appear to be more important (Desar et al., 2010). Small molecular peptides with a better tissue penetration will therefore be less affected by these factors than antibodies. However, a nonspecific uptake into tumor regions still exists. Indeed, although the observed increase of tumor uptake of ^{18}F-Z-3B correlated with the measured levels of hVEGF, the slope of the regression line was low, indicating that the imaging quantification still needs to be improved to accurately reflect VEGF level (Fedorova et al., 2011). Thus, along with the development of probes with optimal specificity and affinity, further improvements in sensitivity and spatial/temporal resolution of the used imaging techniques and advanced quantification algorithm and models are required to thoroughly decipher the images.

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Extending Optogenetics to a Ca^{2+}-Selective Channel

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Many cellular processes are regulated by Ca^{2+} signaling. In this issue of *Chemistry & Biology*, Pham et al. have developed a photo-activated protein, LOVS1K, which enables the generation of local or global Ca^{2+} signals through binding to the Ca^{2+}-specific membrane channel Orai.

Ca^{2+} regulates a variety of physiological processes, including secretion, contraction gene transcription, cell proliferation, and migration (Berridge et al., 2003). A primary Ca^{2+} entry pathway in nonexcitable cells is determined by the Ca^{2+} release activated Ca^{2+} channel. Their two limiting molecular components include the Ca^{2+}-sensor protein stromal interaction molecule 1 (STIM1) that is located in the endoplasmic reticulum and the Orai channel in the plasma membrane (Hogan et al., 2010). STIM1 senses the luminal Ca^{2+} content, in that store depletion induces STIM1 oligomerisation into punctalike structures. This in turn induces the coupling of the cytosolic C-terminal domain of STIM1 with Orai1, thereby triggering channel opening and Ca^{2+} entry (Fahrner et al., 2009).

In the current issue of *Chemistry & Biology*, Pham et al. (2011) present a method that enables control of Ca^{2+} entry through Orai1 channels via a photo-activated STIM1 protein. They utilized the LOV2 domain of phototropin-1 to generate a photo-activated switch (Strickland et al., 2008; Wu et al., 2009) in a fusion protein of LOV2 that is N-terminally linked to a cytosolic fragment of STIM1 (amino acids 233–450), which they termed LOVS1K. Upon a 300 ms exposure to blue light, LOVS1K relocates within seconds from the cytosol onto Orai1. This translocation is reversible within 10–30 s in the dark, leading to LOVS1K dissociation from Orai1 in the plasma membrane, thus allowing for repeated recruitment by subsequent photo-stimulation.

The mechanism of this light-induced LOVS1K relocation, as suggested by the authors in their model, may involve a conformational rearrangement within the LOV2 domain that reduces the sterical hindrance that prevents the interaction of LOVS1K with Orai1 in the dark state. Sterical shielding has recently been also reported (Yu et al., 2011) for residues

